

# Carbohydrates

Integrated Research on  
Glycobiology and Glycotechnology

Volume I

Sydney Marsh



# Carbohydrates: Integrated Research on Glycobiology and Glycotechnology Volume I

Edited by Sydney Marsh



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Volume I**  
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**Carbohydrates: Integrated Research on  
Glycobiology and Glycotechnology**

**Volume I**

## Preface

This book has been compiled for those interested in the study of carbohydrates. It has many topics which have been addressed by experts from diverse disciplines of microbiology, chemistry, botany, zoology and biotechnology. It encompasses the fundamentals of carbohydrates along with the tools, technologies and experiences for those who are involved in glycobiology and related fields. The book covers organic reactions of carbohydrates, analysis of carbohydrate derivatives, studies of DC-SIGN antagonists and the biosynthesis of carbohydrates in a microorganism. This is a comprehensive book which would cater to the needs of different kinds of readers.

The information shared in this book is based on empirical researches made by veterans in this field of study. The elaborative information provided in this book will help the readers further their scope of knowledge leading to advancements in this field.

Finally, I would like to thank my fellow researchers who gave constructive feedback and my family members who supported me at every step of my research.

**Editor**

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**List of Contributors**

# Chemistry and Biochemistry

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# Carbohydrate Microarray

Chuan-Fa Chang

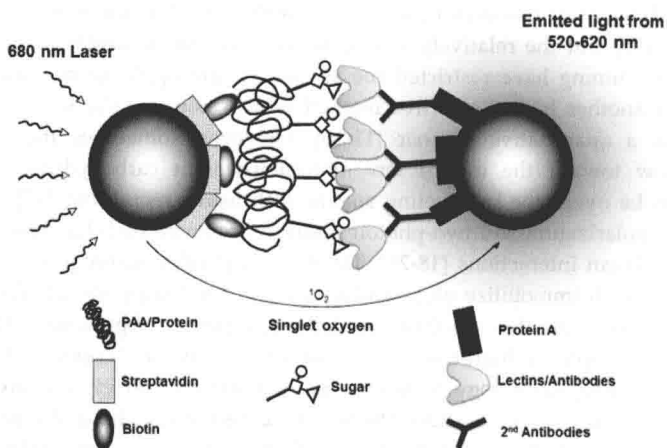
Additional information is available at the end of the chapter

## 1. Introduction

Glycosylation adorns more than one half of the proteins in eukaryotic cells [1,2]. This post-translational modification plays an indispensable role in many important biological events, especially on cell surface [1,3]. Alterations in carbohydrate structures are known to correlate with the changes in protein stability and clearance, as well as various physiological functions including cell-cell adhesion, inflammation, tumor metastasis, and infection of bacteria and viruses [4-8]. Although glycosylation is essential for the formation and progression of various diseases, study of this subject is hampered by lack of effective tools available to date, in addition to structural heterogeneity and complexity of carbohydrates. A number of techniques have been developed to analyze the binding interactions between carbohydrates and proteins [2,9]. For instances, lectin blotting/binding assay has become a routine method to determine the glycan-protein interactions [10], but the relatively low sensitivity and the necessity of multiple wash steps/time-consuming have restricted the sensitivity and application. Surface plasmon resonance is another highly sensitive method which monitors the interactions in real time and in a quantitative manner [11-16]. However, sometimes the sensitivity is relatively low toward the use of low molecular-weight carbohydrates, though the problem can be overcome by labeling sugars with heavy metal ions [17]. In addition, fluorescence polarization and two-photon fluorescence correlation have been applied to study lectin-glycan interactions [18-21]. The most applicable technique is carbohydrate microarrays which immobilize oligosaccharides to a solid supports are developed and widely used to measure the carbohydrate binding properties of proteins, cells, or viruses [22-25]. For example, a high-content glycan microarray is developed by a robotic microarray printing technology in which amine-functionalized glycans are coupled to the succinimide esters on glass slides [26,27]. These microarrays have also been subjected for profiling the carbohydrate binding specificities of lectins, antibodies, and intact viruses.

## 2. Carbohydrate microarray

In our recent work, we have developed two novel carbohydrate microarrays: solution microarray [28] and membrane microarray [29]. Carbohydrate solution assay is a high-throughput, homogenous and sensitive method to characterize protein-carbohydrate interactions and glycostructures by in-solution proximity binding with photosensitizers (Figure 1). The technology, also called AlphaScreen™, is first described by Ullman et al., and has been used to study interactions between biomolecules [30-34]. In these assays, a light signal is generated when a donor bead and an acceptor bead are brought into proximity. This method usually provides good sensitivity with *femto*-mole detection under optimized conditions, relying on the binding affinity between analytes. All the procedures are carried out in 384-welled microtiter plates, thus qualifying the protocol as high-throughput. Two particles of 200 nm are involved in this technology including streptavidin-coated particles (donor beads) and protein A-conjugated particles (acceptor beads). Biotinylated polyacrylamide (biotin-PAA)-based glycans that are immobilized on donor beads can be recognized by lectins or antibodies, and connected with acceptor beads through specific antibodies (Figure 1). A number of carbohydrate binding proteins, including eleven lectins and seven antibodies, are profiled for their carbohydrate binding specificity to validate the efficacy of this developed technology. This assay is performed in homogeneous solutions and does not require extra wash steps, preventing the loss of weak bindings that often occur in the repeating washes of glycan microarray. However, antigen/ligand excess effect may happen in the homogeneous solution assay if the concentrations of carbohydrate epitopes, proteins, or antibodies are too high. One mg of biotin-PAA-sugar can be applied for fifty thousand assays because minimal amount of materials are needed in this microarray system (a range of nano-gram is required per well). Although the detection limit of biotin-PAA-sugar is good (2 ng per well), the linear range is too narrow for quantitative application.



**Figure 1.** In-solution proximity binding with photosensitizers which was developed to characterize the protein-carbohydrate interactions [28].

Carbohydrate membrane microarray is fabricated by immobilization of the biotin-conjugated PAA-based glycans on aldehyde-functionalized UltraBind via streptavidin. Streptavidin interacted strongly with biotin and formed covalent linkage with membranes after reductive amination, which prevented the loss of glycans from membrane during repeated wash steps. The use of PAA also avoided the nonspecific interactions that take place in other studies between some lectins and non-glycosylated proteins (e.g. HAS or BSA) [35]. The operation of this carbohydrate membrane microarray is similar to that of Western blotting and can be performed easily by anyone without prior intensive training.

### 3. Applications

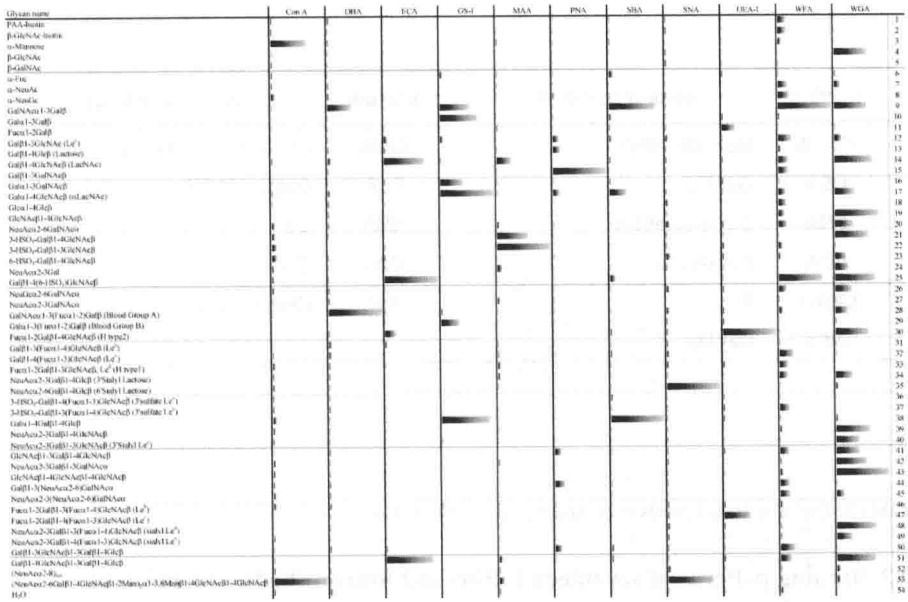
#### 3.1. Carbohydrate binding profiles of lectins and antibodies (solution microarray)

Fifty-four biotinylated polyacrylamide backbone glycans (biotin-PAA-glycans) (Table 1) are collected in total to examine fifteen carbohydrate-binding proteins, including eight lectins (Con A, DBA, GS-I, PNA, SBA, UEA-1, WFA and WGA), and six antibodies (anti-Le<sup>a</sup>, Le<sup>b</sup>, Le<sup>x</sup>, Le<sup>y</sup>, sialyl Le<sup>a</sup> and sialyl Le<sup>x</sup>). The resulting signals are indicated with bars as relative intensities (Figures 2 and 3). The natural carbohydrate ligands for these lectins are listed in Table 2. All of the lectins showed nearly the same carbohydrate binding preferences as those in literatures. For example, concanavalin A (Con A) bound preferentially to mannose (No. 3) and biantennary *N*-glycan (No. 53), and very weakly to 3- and 6-sulfated galactosides (No. 19, 23 and 25). DBA, a GalNAc-binding lectin, recognized GalNAc $\alpha$ 1-3Gal-containing epitopes (No. 11 and 39). ECA interacted with LacNAc disaccharide, Gal $\beta$ 1-4(6-sulfo)GlcNAc, and Gal $\beta$ 1-4( $\alpha$ 1-2Fuc)GlcNAc (No. 17, 24, 31 and 47), and weakly bound to Le<sup>c</sup> (Gal $\beta$ 1-3GlcNAc, No. 20). GS-I preferred interacting with Gal/GalNAc that contains  $\alpha$ 1-3 or 1-4 linkage (No. 11, 13, 14, 16, 40 and 42). MAA, in this study, recognized mainly to 3'-sulfated Gal $\beta$ 1-3GlcNAc, 3'-sulfated Gal $\beta$ 1-4GlcNAc and LacNAc and weakly to 3-sialylated galactosides (No. 26, 37 and 53). PNA interacted with Gal $\beta$ 1-3GalNAc (No. 15) and bound to some galactosides weakly (No. 12, 16, 20, 45 and 46). SBA preferentially interacted with  $\alpha$ -linked galactosides (No. 16 and 42) and *N*-acetylgalactosaminoside (No. 11). SNA, a well-known  $\alpha$ 2-6 sialoside-binding lectin, interacted strongly to 6'-sialyl lactose and sialylated diantennary *N*-glycan (No. 36 and 53). UEA-1 specifically bound with Fuca1-2Gal-containing glycans (No. 18, 31 and 49). Due to weak interaction with PAA, WFA is the only one lectin showing higher background signals than the others. It recognized nearly half of the glycans on the glycan library, such as GlcNAc- and NeuAca2-3-Gal/NeuAca2-6-Gal containing saccharides. WGA also bound to terminal Gal or GalNAc epitopes (GalNAc $\alpha$ 1-3Gal, No. 11 and Gal $\beta$ 1-4(6HSO<sub>3</sub>)GlcNAc, No. 24) according to some minor signals. Interestingly, WGA showed better interactions with chitotriose than with chitobiose and GlcNAc. In addition, the binding specificities of monoclonal anti-carbohydrate antibodies also revealed some interesting features. As shown in Figure 3, anti-Le<sup>a</sup> antibody bound tightly with Le<sup>x</sup>, but less with Le<sup>b</sup> and sialyl Le<sup>a</sup>. Anti-Le<sup>b</sup> antibody represented specificities for both Le<sup>b</sup> and

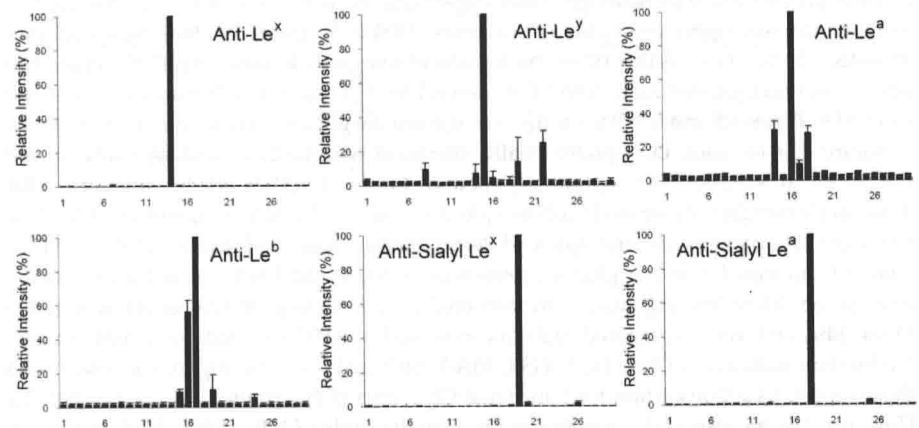
Le<sup>a</sup>, but less for Le<sup>x</sup> and sialyl Le<sup>x</sup>. Anti-Le<sup>y</sup> antibody not only binding to Le<sup>y</sup>, but also recognized lactose, Le<sup>x</sup>, sialyl Le<sup>x</sup> and H type 2 structures. We also compared the binding patterns of lectins with the results reported by Blixt and coworkers at CFG in which 264 different glycans are studied by using the printed microarray (Ver. 2) (<http://www.functionalglycomics.org/glycomics/publicdata/primaryscreen.jsp>). There are forty-seven glyco-epitopes are found to be identical in both analyses. Even the different principles and procedures of the two systems, the binding patterns of eight lectins are nearly the same, except for a few minor differences. For example, our characterized patterns of WFA and WGA show 90% similarity to the CFG data. Nevertheless, the interactions of SBA, WFA and WGA to  $\beta$ -GalNAc (No. 2) in the CFG's printed microarray are not observed in our system. Both of our method and the printed microarray indicate that MAA preferentially binds to sulfated glycans [36]. Because of the observed consistency shown by the two very different methods, we conclude the protein-glycan binding interactions are not affected by the PAA linker, the assay procedure (washing vs. non-washing) and the interacting microenvironment (2D for printed microarray vs. 3D for our solution microarray).

No.	Glycan Name	No.	Glycan Name
1	PAA-biotin	28	GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$ . sp <sup>==</sup> -NHCOCH2NH-
2	$\beta$ -GlcNAc	29	GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$
3	$\alpha$ -Mannose	30	Fuc $\alpha$ 1-2Gal $\beta$ 1-3GlcNAc $\beta$ . Le <sup>a</sup> (H type1)
4	$\beta$ -GlcNAc	31	Fuc $\alpha$ 1-2Gal $\beta$ 1-4GlcNAc $\beta$ (H type2)
5	$\beta$ -GalNAc	32	Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc $\beta$ (Le <sup>a</sup> )
6	$\alpha$ -Fuc	33	Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ (Le <sup>a</sup> )
7	$\alpha$ -NeuAc	34	3-HSO <sub>3</sub> -Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ (3'sulfate Le <sup>a</sup> )
8	$\alpha$ -NeuGc	35	NeuAc $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ (3'Sialyl Le <sup>a</sup> )
9	Glc $\alpha$ 1-4Glc $\beta$	36	NeuAc $\alpha$ 2-6Gal $\beta$ 1-4Glc $\beta$ (6'Sialyl Lactose)
10	GlcNAc $\beta$ 1-4GlcNAc $\beta$	37	NeuAc $\alpha$ 2-3Gal $\beta$ 1-4Glc $\beta$ (3'Sialyl Lactose)
11	GalNAc $\alpha$ 1-3Gal $\beta$	38	NeuAc $\alpha$ 2-3(NeuAc $\alpha$ 2-6)GalNAc $\alpha$
12	Gal $\beta$ 1-4Glc $\beta$ (Lactose)	39	GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ (Blood Group A)
13	Gal $\alpha$ 1-3Gal $\beta$	40	Gal $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ (Blood Group B)
14	Gal $\alpha$ 1-3GalNAc $\beta$	41	3-HSO <sub>3</sub> -Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc $\beta$ (3'sulfate Le <sup>a</sup> )
15	Gal $\beta$ 1-3GalNAc $\beta$	42	Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc $\beta$
16	Gal $\alpha$ 1-4GlcNAc $\beta$ ( $\alpha$ LacNAc)	43	NeuAc $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$
17	Gal $\beta$ 1-4GlcNAc $\beta$ (LacNAc)	44	NeuAc $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$
18	Fuc $\alpha$ 1-2Gal $\beta$	45	Gal $\beta$ 1-3(NeuAc $\alpha$ 2-6)GalNAc $\alpha$
19	3-HSO <sub>3</sub> -Gal $\beta$ 1-4GlcNAc $\beta$	46	Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$
20	Gal $\beta$ 1-3GlcNAc (Le <sup>a</sup> )	47	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$
21	NeuAc $\alpha$ 2-6GalNAc $\alpha$	48	Fuc $\alpha$ 1-2Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc $\beta$ (Le <sup>b</sup> )
22	NeuGc $\alpha$ 2-6GalNAc $\alpha$	49	Fuc $\alpha$ 1-2Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ (Le <sup>a</sup> )
23	3-HSO <sub>3</sub> -Gal $\beta$ 1-3GlcNAc $\beta$	50	NeuAc $\alpha$ 2-3Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc $\beta$ (sialyl Le <sup>a</sup> )
24	Gal $\beta$ 1-4(6-HSO <sub>3</sub> )GlcNAc $\beta$	51	NeuAc $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ (sialyl Le <sup>a</sup> )
25	6-HSO <sub>3</sub> -Gal $\beta$ 1-4GlcNAc $\beta$	52	(NeuAc $\alpha$ 2-8) <sub>s,c</sub>
26	NeuAc $\alpha$ 2-3Gal	53	(NeuAc $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man) <sub>2</sub> $\alpha$ 1-3,6Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$
27	NeuAc $\alpha$ 2-3GalNAc $\alpha$	54	H <sub>2</sub> O

**Table 1.** List of biotin-PAA-glycans (fifty-two) used in glycan solution microarray [28].



**Figure 2.** Carbohydrate binding specificities of eleven lectins characterized by glycan solution microarray [28].



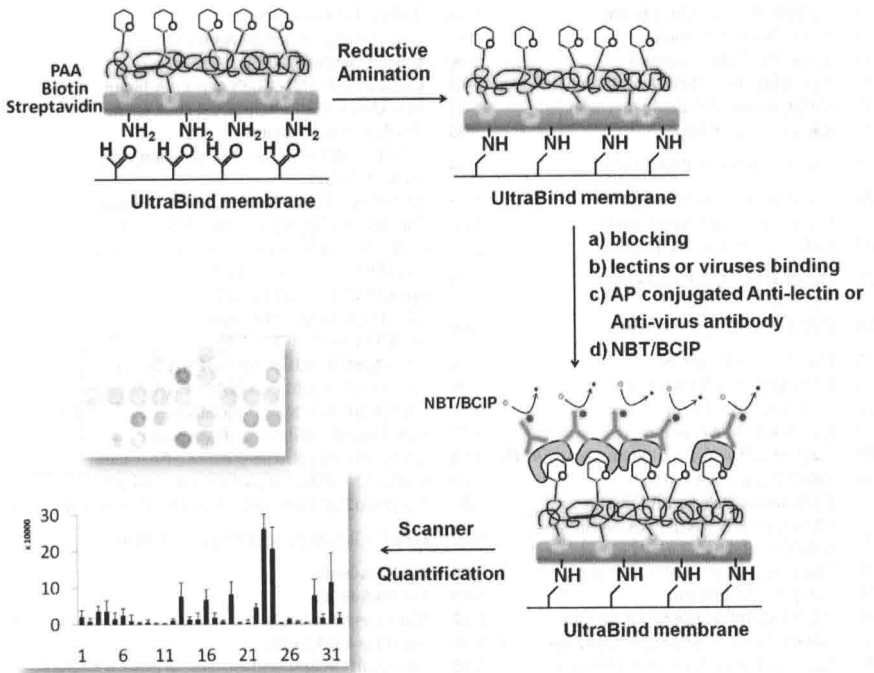
**Figure 3.** Carbohydrate binding specificities of six antibodies characterized by glycan solution microarray [28].

Lectins	Binding specificities	Lectins	Binding specificities
<b>Con A</b>	Man, Glc, GlcNAc	<b>WGA</b>	GlcNAc $\beta$ 1-4GlcNAc, Neu5Ac
<b>DBA</b>	GalNAc	<b>ECA</b>	Gal $\beta$ 1-4GlcNAc
<b>PNA</b>	Gal $\beta$ 1-3GalNAc	<b>MMA</b>	Gal
<b>SBA</b>	GalNAc/Gal	<b>GS-I</b>	Gal
<b>UEA-1</b>	Fuc	<b>SNA</b>	Neu5Ac $\alpha$ 2-6
<b>WFA</b>	GalNAc		

**Table 2.** Carbohydrate ligands of commercial available lectins.

### 3.2. Binding patterns of seventeen lectins and four antibodies (membrane microarray)

The principle and procedures of carbohydrate membrane microarray are showed in **Figure 4**. The western blotting like procedures not only reduces the time and interference, but also increases the application of this platform. In order to look deep inside the carbohydrate binding preferences of proteins and microorganisms, the collections of biotin-PAA-glycans were to increased eighty-eight different structures (**Table 3**). The glycan binding specificities of sixteen lectins (six alkaline phosphatase (AP)-conjugated lectins, four FITC-conjugated lectins, six unconjugated lectins) and four Lewis blood-group antibodies are evaluated and showed in **Figures 5 and 6**. All the lectins recognized the glycans that are consistent with the literature. For instance, ECA preferentially interacted with LacNAc, lactose, GalNAc, and Gal terminal sugars; PNA specifically bound to the Gal $\beta$ 1-3GalNAc structure; SBA dominantly recognized  $\alpha$ -linked GalNAc epitopes; 3-sulfate LacNAc is ligand for MAA [36]. Compare the patterns of unconjugated lectins with conjugated lectins (AP- or FITC-attached) indicated that the glycan preferences of ECA and PNA are not interfered by conjugation. More binding signals are observed in the binding profiles of AP-conjugated MAA, SBA and WGA compared with unconjugated or FITC-attached ones. Additionally, the binding patterns of DBA, ECA, GS-I, MAA, SBA and VVA are highly consistent with those reported by Consortium for Functional Glycomics (CFG, printed microarray Ver. 2.). However, few inconsistencies are also observed in the study of MPA, PNA, UEA and WGA. Furthermore, the binding patterns of four Lewis blood group antibodies represented very high specificities (**Figure 6**).



**Figure 4.** Fabrication, principle, and procedures of carbohydrate membrane microarray [29].



S-1	Blank-PAA-biotin	S-46	Neu5Ac <sub>2</sub> -6Gal $\beta$ -PAA-biotin
S-2	$\beta$ -GlcNAc-sp-biotin	S-47	Neu5Gc <sub>2</sub> -6GalNAc-PAA-biotin
S-3	$\alpha$ -Mannose-PAA-biotin	S-48	Neu5Ac <sub>2</sub> -3GalNAc $\alpha$ -PAA-biotin
S-4	$\beta$ -GlcNAc-PAA-biotin	S-49	Blood Group A-tri-PAA-biotin
S-5	$\beta$ -GalNAc-PAA-biotin	S-50	Blood Group B-tri-PAA-biotin
S-6	$\alpha$ -L-Fuc-PAA-biotin	S-51	H(type2)-PAA-biotin
S-7	$\alpha$ -Neu5Ac-PAA-biotin	S-52	Le <sup>x</sup> -PAA-biotin
S-8	$\alpha$ -Neu5Ac-OCH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> -p-NHCOOCH <sub>2</sub> -PAA-biotin	S-53	Le <sup>x</sup> -PAA-biotin
S-9	MDP(muramyl dipeptide)-PAA-biotin	S-54	Le <sup>a</sup> (H type1)-PAA-biotin
S-10	$\alpha$ -Neu5Gc-PAA-biotin	S-55	3'Sialyl-Lactose-PAA-biotin
S-11	$\beta$ -D-Gal-3-sulfate-PAA-biotin	S-56	6'Sialyl-Lactose-PAA-biotin
S-12	$\beta$ -D-GlcNAc-6-sulfate-PAA-biotin	S-57	3-HSO <sub>3</sub> -Le <sup>x</sup> -PAA-biotin
S-13	GalNAc $\alpha$ -3Gal $\beta$ -PAA-biotin	S-58	3-HSO <sub>3</sub> -Le <sup>a</sup> -PAA-biotin
S-14	Gal $\alpha$ 1-3Gal $\beta$ -PAA-biotin	S-59	Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc $\beta$ -PAA-biotin
S-15	Fuca1-2Gal $\beta$ -PAA-biotin	S-60	Gal $\alpha$ 1-3Gal $\beta$ 1-4Glc $\beta$ -PAA-biotin
S-16	Le <sup>c</sup> (Gal $\beta$ 1-3GlcNAc)-PAA-biotin	S-61	GlcNAc $\beta$ 1-2Gal $\beta$ 1-3GalNAc $\alpha$ -PAA-biotin
S-17	Gal $\beta$ 1-4Glc $\beta$ -PAA-biotin (Lactose)	S-62	Neu5Ac <sub>2</sub> -3Gal $\beta$ 1-4GlcNAc $\beta$ -PAA-Biotin
S-18	LacNAc-PAA-biotin	S-63	3'Sialyl-Le <sup>a</sup> -PAA-biotin
S-19	Fuca1-3GlcNAc $\beta$ -PAA-biotin	S-64	Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ -PAA-biotin, sp=NHCOCH <sub>2</sub> NH-
S-20	Fuca1-4GlcNAc $\beta$ -PAA-biotin	S-65	GlcNAc $\alpha$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$ -PAA-biotin
S-21	GalNAc $\alpha$ 1-3Gal $\beta$ -PAA-biotin	S-66	GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$ -PAA-biotin
S-22	Gal $\alpha$ 1-3GalNAc $\alpha$ -PAA-biotin	S-67	Gal $\beta$ 1-3(GlcNAc $\beta$ 1-6)GalNAc $\alpha$ -PAA-biotin
S-23	Gal $\beta$ 1-3GalNAc $\beta$ -PAA-biotin	S-68	Blood type A (tri)-PAA-biotin, sp=(CH <sub>2</sub> ) <sub>3</sub> NHCO(CH <sub>2</sub> ) <sub>3</sub> NH-
S-24	Gal $\alpha$ 1-3GalNAc $\beta$ -PAA-biotin	S-69	Blood type B (tri)-PAA-biotin, sp=(CH <sub>2</sub> ) <sub>3</sub> NHCO(CH <sub>2</sub> ) <sub>3</sub> NH-
S-25	Gal $\beta$ 1-3Gal $\beta$ -PAA-biotin	S-70	GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc-PAA-biotin
S-26	GlcNAc $\beta$ 1-3Gal $\beta$ -PAA-biotin	S-71	Neu5Ac <sub>2</sub> -3Gal $\beta$ 1-3GalNAc $\alpha$ -PAA-Biotin
S-27	$\alpha$ LacNAc-PAA-biotin	S-72	GlcNAc $\beta$ 1-3(GlcNAc $\beta$ 1-6)GalNAc $\alpha$ -PAA-biotin
S-28	Glc $\alpha$ 1-4Glc $\beta$ -PAA-biotin	S-73	Gal $\alpha$ 1-4Gal $\beta$ 1-4GlcNAc $\beta$ -PAA-biotin
S-29	Gal $\beta$ 1-3GalNAc $\alpha$ -PAA-biotin, sp=p-OC <sub>6</sub> H <sub>4</sub> -	S-74	GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-PAA-biotin
S-30	Gal $\alpha$ 1-2Gal $\beta$ -PAA-biotin	S-75	Gal $\beta$ 1-3(Neu5Ac <sub>2</sub> -6)GalNAc $\alpha$ -PAA-biotin
S-31	GlcNAc $\beta$ 1-4GlcNAc-PAA-biotin	S-76	Neu5Ac <sub>2</sub> -3(Neu5Ac <sub>2</sub> -6)GalNAc-PAA-biotin
S-32	GlcNAc $\beta$ 1-4GlcNAc $\beta$ -PAA-biotin, sp=NHCOCH <sub>2</sub> NH-	S-77	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$ -PAA-biotin
S-33	Neu5Ac <sub>2</sub> -6GalNAc-PAA-biotin	S-78	Le <sup>a</sup> -PAA-biotin
S-34	H(type 3)-PAA-biotin	S-79	Le <sup>x</sup> -PAA-biotin
S-35	3-HSO <sub>3</sub> -Gal $\beta$ 1-4GlcNAc-PAA-biotin	S-80	Sialyl Le <sup>a</sup> -PAA-biotin
S-36	3-HSO <sub>3</sub> -Gal $\beta$ 1-3GlcNAc $\beta$ -PAA-biotin	S-81	Sialyl Le <sup>x</sup> -PAA-biotin
S-37	Gal $\alpha$ 1-6Glc $\beta$ -PAA-biotin (melibiose)	S-82	GlcNAc $\beta$ 1-3(GlcNAc $\beta$ 1-6)Gal $\beta$ 1-4Glc $\beta$ -PAA-biotin
S-38	Neu5Ac <sub>2</sub> -8Neu5Ac $\alpha$ -sp*-PAA-biotin, (Neu5Ac) <sub>2</sub>	S-83	Gal $\alpha$ 1-3(Fuca1-2)Gal $\beta$ 1-4GlcNAc-PAA-biotin
S-39	Gal $\beta$ 1-2Gal $\beta$ -PAA-biotin	S-84	Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ -PAA-biotin
S-40	6-HSO <sub>3</sub> -Gal $\beta$ 1-4GlcNAc-PAA-biotin	S-85	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ -PAA-biotin
S-41	Neu5Ac <sub>2</sub> -3Gal-PAA-biotin	S-86	(NeuAca2-8)5-6-PAA-biotin
S-42	Gal $\beta$ 1-4(6-HSO <sub>3</sub> )GlcNAc $\beta$ -PAA-biotin	S-87	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3(Gal $\beta$ 1-4GlcNAc $\beta$ 1-6)GalNAc $\alpha$ -PAA-biotin
S-43	3-HSO <sub>3</sub> -Gal $\beta$ 1-3GalNAc $\beta$ -PAA-biotin (sulfate-TF)	S-88	$\alpha$ 2-6 sialylated diantennary N-glycans-PAA-biotin
S-44	GlcNAc $\beta$ 1-3GalNAc $\alpha$ -PAA-biotin	S-89	GalNAc $\alpha$ -Ser-PAA-biotin
S-45	GlcNAc $\beta$ 1-6GalNAc $\alpha$ -PAA-biotin	S-90	H <sub>2</sub> O

$\alpha$ 2-6 sialylated diantennary N-glycans :(NeuAca2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man) $\alpha$ 1-3,6Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc

**Table 3.** List of biotin-PAA-glycans (eighty-eight) used in carbohydrate membrane microarray [29].